EVOLUTIONARY AND ECOLOGICAL RELATIONSHIPS OF GILL SLIT MORPHOLOGY IN EXTANT SHARKS

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ABSTRACT. One of the defining characteristics of Elasmobranchii is the external gill slits, through which water is expelled during exhalation. The morphology of the gill slits is presumed to vary among elasmobranchs, between ram and suction ventilators, and between pelagic and benthic species. However, the evolution of gill slit morphology and the relationship to behavior and ecology is not clear. The relative length of each slit, relative distance between adjacent pairs of slits, and number of slits positioned over the pectoral fin were quantified in 289 preserved specimens representing 185 species in 69 genera, 28 families, and 8 orders of sharks. Six character states of relative gill slit length, five character states of relative inter-gill slit spacing, and five gill slit positions over the pectoral fin were identified. Character mapping onto a phylogeny reveals that the basal state for extant Selachii is to possess slits of similar relative length and spacing with no slits over the pectoral fin. Multiple character states in various combinations evolved within the Carcharhiniformes, which is the most speciose extant group. Ontogenetic differences in gill slit morphology were found in the 12 genera in which adult and juvenile data were collected. Slit spacing in juveniles has an equal tendency to become more similar or more different in adults, whereas slit length changes more often from more similar in juveniles to increasingly different in adults. Exploring the relationships among gill slit morphology and ventilation mode in modern sharks reveals character complexes that are common to four body types. Macropelagic types are ram ventilators with a longer first slit length, similar slit spacing in most, and no slits over the pectoral fin. Most littoral types are ram ventilators, with decreasing slit length and spacing and with one slit over the fin. Benthic types are suction ventilators with decreasing slit length and spacing and up to four slits over the fin. Bathic and micropelagic types are suction ventilators with no slits over the fin and most having similar slit length and spacing. Morphological differences in gill slit morphology may correspond to hydrodynamic differences in exhaled water from the slits by habitat.

Key words: Functional morphology, Ontogeny

INTRODUCTION

Elasmobranchii, the vertebrate group comprising sharks and rays, literally means "plate-gill" and indicates that these fish have five to seven pairs of plates with gill filaments attached. These plates or arches have an external opening or gill slit between each pair through which water taken in through the mouth is expelled during exhalation. The morphology of the gill slits appears to vary anecdotally among elasmobranch taxa, such as between ram- and suction-ventilating species and between pelagic and benthic species (personal observations), but no studies quantify these features and the relationship of gill slit morphology to function, body type, and habitat, and even phylogeny is unknown.

Studies of ventilation in fishes have focused mainly on the mechanics of suction ventilation or physiology of the system. Suction ventilation in fishes is characterized by coordinated movements of the jaws, hyoid arch, gill arches, and gill slits that are responsible for generating pressure differentials in those regions to draw ambient water over the gill filaments (Hughes, 1960; Hughes and Ballintijn, 1965; Ferry-Graham, 1999; Liem et al., 2001; Summers and Ferry-Graham, 2001, 2003). Suction ventilation actively creates a

continuous flow of water over the gill filaments and is used by species that can stop and rest on the substrate (Thomson and Simanek, 1977; Summers and Ferry-Graham, 2001, 2003). In contrast, ram-ventilating species use the forward motion of swimming movements to generate a continuous flow of water over the gill filaments while keeping the mouth, gill arches, and gill slits open (Emery and Szcepanski, 1986; Graham et al., 1990; Parsons and Carlson, 1998; Carlson et al., 1999; Liem et al., 2001; Bernal et al., 2003; Carlson and Parsons, 2003). Large fishes, pelagic fishes, or both, with relatively high oxygen consumption demands, utilize ram ventilation (Thomson and Simanek, 1977; Emery and Szcepanski, 1986; Graham et al., 1990; Parsons and Carlson, 1998; Carlson et al., 1999; Bernal et al., 2003; Carlson and Parsons, 2003).

Modeling studies show that slit size and number, as well as the shape of the oral cavity, affect flow patterns during ram filter feeding (Cheer et al., 2001; Paig-Tran et al., 2011). Increasing the number of gill slits from two to five results in increasing turbulence at the esophagus and a more even distribution of food particles captured across all slits (Paig-Tran et al., 2011). Water velocity is highest when exiting the posteriormost gill slit compared with more anterior slits in cylinder- and cone-shaped oral cavities (Cheer et al., 2001). A higher volume of flow rate also exits the posterior gill slit in cylindrical oral cavities, whereas a higher volume of flow rate occurs at the anteriormost gill slit in conical oral cavities (Cheer et al., 2001).

Some elasmobranch species have been reported to switch between suction on the substrate and ram when swimming; however, this is not well documented, nor is it clear how prevalent is the ability. Leopard sharks, *Triakis semifasciata* (Triakidae), reportedly transition from suction to ram ventilation with increasing swimming speed (Graham et al., 1990). However, several suction-ventilating elasmobranch species swimming steadily in a flow tank from 0.5 to 2.0 body lengths per second (changed incrementally by 0.5 units) did not transition from suction to ram ventilation (unpublished observations of *Squalus acanthias, Triakis semifasciata, Mustelus canis,* and *Chiloscyllium plagiosum*). Rather, they stopped swimming and continued to suction ventilate with increased gape and branchial expansion to pass more water over the gills. Certainly, forward motion is likely to assist suction ventilation when swimming, but we have not observed any species switching from suction to ram at higher swimming speeds (personal observations). Obligate ram ventilators like sandbar, *Carcharhinus plumbeus*, and bonnethead, *Sphyrna tiburo*, sharks will attempt to buccal pump when prevented from swimming but soon lose righting ability and presumably will suffocate if not allowed to resume swimming behavior (personal observation). More study is needed to understand the circumstances and prevalence of the ability to transition between ventilation modes.

The number of gill arches and slits possessed by shark species is usually noted for taxonomic classification (Compagno, 1984). Sharks of the order Hexanchiformes possess six or seven pairs of gill arches and slits, and some species from the order Pristiophoriformes have six pairs of gill arches and slits; the remaining shark species possess five pairs of gill arches and slits (Compagno, 1984). Yet, other character state differences in gill slit morphology are discussed anecdotally in some shark taxa (Compagno, 1984) and are apparent with a preliminary qualitative investigation across a broad range of species. For example, the gill slits of Port Jackson sharks, *Heterodontus portjacksoni,* (Heterodontiformes) appear to decrease in length from the anterior to posterior direction such that the first pair of slits is noticeably longer than the fifth pair (Grigg, 1970). The slits of many lamniform species are considered to be "moderately long" to "long," and the fifth gill slit in squalid species may be "enlarged" (Compagno, 1984). Similar observations have been made about the relative spacing
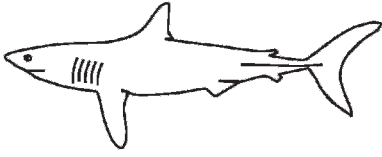
A) Body type 1 - Macropelagic Ecomorphotypes
Taxa: Cetorhinidae and Lamnidae
Ventilation: obligate ram
Features: high aspect ratio caudal fin, lateral keel
Habitat: oceanic cruisers

B) Body type 2 - Littoral Ecomorphotypes
Taxa: Mitsukurinidae, Odontaspididae, Alopidae, Carcharhinidae
Ventilation: obligate ram and suction, some transition
Features: lower aspect ratio caudal fin, flattened ventral head surface, generalized morphology
Habitat: continental cruisers

C) Body type 3 – Benthic Ecomorphotypes
Taxa: Heterodontiformes, Orectolobiformes, Carcharhiniformes except Carcharhinidae, Hexanchiformes and Pristiophoriformes
Ventilation: primarily suction, some ram and transition
Features: low caudal fin angle, small or no hypocaudal lobe (H), more anterior pelvic fins
Habitat: benthic

D) Body type 4 – Bathic and Micropelagic Ecomorphotypes
Taxa: Squaliformes
Ventilation: suction
Features: no anal fin, large epicaudal lobe (E), high lateral pectoral fins
Habitat: littoral, micropelagic, or bathic

Figure 1.   Body types in sharks (after Thomson and Simanek, 1977).

between adjacent gill slits (Compagno, 1984). For example, the fourth pairs of interbranchial septa appear to overlap the fifth pair in orectolobiform species (Compagno, 1984). The position of the gill slits relative to the pectoral fin in some major groups has also been noted for taxonomic purposes (Compagno, 1984). The posterior two or three pairs of gill slits are positioned over the pectoral fin in orectolobiform and heterodontiform species, whereas the slits of most lamniform species are positioned anterior to the pectoral fin (Compagno, 1984). However, no quantitative studies exist documenting morphological differences that may have phylogenetic or ecological implications among shark taxa.

Several body types have been proposed for sharks that may also have correlations to ventilation mode and morphology. These shark body type categories and ecomorphotypes (groups sharing similar morphology, habitat, and behavior) have been simplified into four groups that are useful in inferring ecological and behavioral niche (Thomson and Simanek, 1977; Compagno, 1990; Wilga and Lauder, 2004), noting that exceptions may well occur: macropelagic, littoral, benthic, and micropelagic/bathic (Fig. 1). Gill slit morphology, body type, and ecomorphotypes can be mapped onto existing phylogenies to evaluate evolutionary and ecological trends in gill slit morphology in sharks. Ontogenetic changes in gill slit structure may also reflect body and habitat changes that sometimes occur over the life history of sharks.

It is clear that gill slit morphology has several character states among sharks. However, the relationship of gill slit morphology to phylogeny (other than number of slits or arches), ventilation behavior, ontogeny, and habitat preference is not clear. Comparisons of gill slit morphology across a broad range of shark species will clarify these relationships. Therefore, the goals of this study are to: 1) identify the variety of character states in gill slit morphology across a wide range of extant
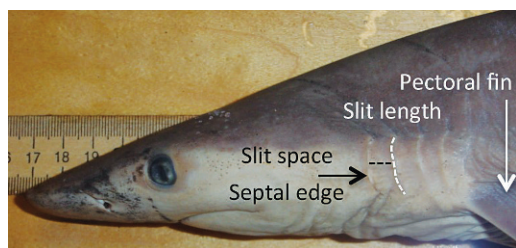
Figure 2. Measurements of gill slit characters on a representative shark with five pairs of slits. Length taken as the total length of the edge of the interbranchial septum as shown by the white vertical dashed line. Inter-slit distance taken as the distance between adjacent pairs of gill slits at the midpoint as shown by the black horizontal dashed line. In this example, slit 5 is positioned over the pectoral fin.

shark species, 2) investigate evolutionary trends in gill slit morphology, 3) investigate relationships between body form, ventilation behavior, and habitat to gill slit morphology, and 4) assess ontogenetic changes in gill slit morphology.

MATERIALS AND METHODS

Measurements were made on 289 preserved specimens representing 185 species in 69 genera, 28 families, and 8 orders of sharks. Specimens are housed in collections at the Museum of Comparative Zoology (MCZ) at Harvard University, the American Museum of Natural History (AMNH), the California Academy of Sciences (CAS), or the University of Rhode Island collection (URI) (Supplemental Appendix 1). Adult and juvenile specimens were used, and maturity was determined by total length (Compagno, 1984). If mature lengths were not available, length at maturity was determined by using the life history tool in FishBase (Froese and Pauly, 2013) that uses empirical relationships to estimate length at maturity from asymptotic and maximum observed lengths (see Froese and Binohlan, 2000).

The following characters of gill slit morphology were measured on each specimen: 1) gill slit length as the length of the slit along the dorsal to ventral septal edge and 2) inter-gill slit distance as the distance between adjacent slits from one septal edge

to the next at the midpoint along the height of the slits (Fig. 2). Measurements were taken unilaterally from the left side of the animal unless the left side was not fit for measurement (after Compagno, 1984). The number of slits positioned completely over the anterior border of the pectoral fin was counted (Fig. 2) and total length measured.

Characters

Characters of gill slit morphology were mapped onto an existing cladogram compiled after Naylor (1992), Shirai (1996), Lopez et al. (2006), Douady et al. (2003), Goto (2001), and Human et al. (2006). The methods of Wiley et al. (1991) were used to map characters and infer morphological state at the nodes. Gill slit characters from this study were placed at the tips of the branches. Characters were then coded at the nodes by interpreting and polarizing characters starting with the most distant branches by comparing outgroups according to phylogenetic parsimony criteria (Wiley et al., 1991). Each decision was made on a single transformation series at a time, following rules for determining overall parsimony and relative apomorphy (Wiley et al., 1991). These analyses were conducted to understand how gill slit characters may have evolved among sharks and to evaluate whether morphological correlates to body and ecomorphotypes are phylogentically based.

Statistical Analyses

Only one individual of many species was available in the museum collections; therefore, species were grouped by genus for statistical analyses to identify differences in morphology among gill slits within genera. When only one individual was available within any genus, it was not used in the analysis. Thus analyses were made on 273 preserved specimens representing 169 species in 50 genera, 24 families, and 8 orders of sharks.

The specimens compared statistically in this study (those in a genus with more than one specimen) spanned a wide range of total lengths (15.0–487.7 cm, Supplementary

Appendix[1]); therefore, the data were standardized (see below) to account for scaling differences. The relative differences in gill slit character states within genera were the dependent variables of interest, so each gill slit length was standardized to the greatest slit length within each individual. The length of each gill slit within an individual was represented as a fraction of the longest slit. For instance, if slit lengths were 2, 3, 4, 5, and 6 cm long within an individual, all of the slits were divided by 6 for standardized values of 0.33, 0.50, 0.67, 0.83, and 1.0. Similarly, each inter-gill slit distance was standardized to the greatest distance between adjacent slits within each individual and represented as a fraction of the greatest distance. Ratios were arcsine transformed to achieve normality when the data were not normally distributed (Zar, 1996).

One-way analysis of variance (*F*-statistic) and Tukey multiple comparisons tests were used to test for differences in relative gill slit length and inter-gill slit distances within genera that had normal distributions. Kruskal–Wallis analyses of variance on Ranks (*H*-statistic) was used to test for differences within genera on arcsine-transformed data that still had nonnormal distributions. SigmaPlot (Vers.11, Systat) was used to run the analyses of variance. A principal components analysis on the correlation matrix was conducted to explore relationships among the variables using SAS (v.9.2). Gill slit length and spacing were coded as follows: 1, sequentially increasing (I, I2); 2, last slit longer (L5); 3, similar (S); 4, first slit longer (L1) or last slit shorter (S4 spacing or S5 length); 5, sequentially decreasing (D, D2). Number of gill slits over the fin was coded as the number. Body types were coded as described above: 1, oceanic; 2, littoral; 3, benthic; 4, bathic/micropelagic.

Character Mapping

The cladogram used here is based on morphological and molecular data and was

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[1] Supplemental material referenced in this paper is available online at http://www.mcz.harvard.edu/Publications.
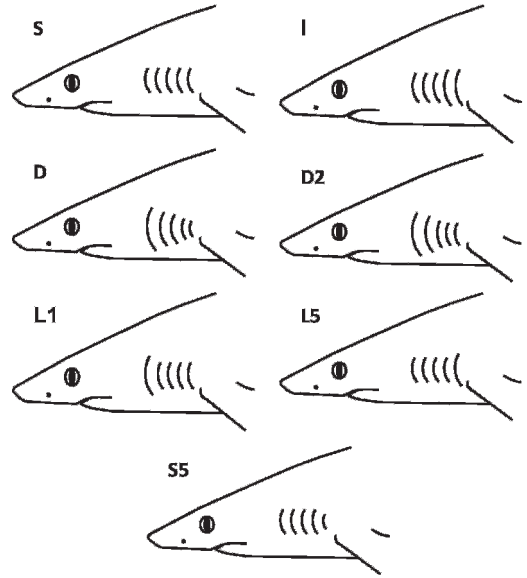

Figure 3. Schematics illustrating relative gill slit length character states. S, similar lengths for all slits; I2, slit length increases in the anterior to posterior direction in two groupings (123<45); D, slit length decreases in the anterior to posterior direction in three or more groupings (1>23>45); D2, slit length decreases in the anterior to posterior direction in two groupings (12>345); L1, slit 1 is longer than all other slits; L5, slit 5 is longer than all other slits; S5, slit 5 is shorter than all other slits.

compiled after Naylor (1992), Shirai (1996), Goto (2001), Douady et al. (2003), Human et al. (2006), and Lopez et al. (2006). It is presumed that selachians form a monophyletic group with all descendants arising from a common ancestor (Shirai, 1996; Douady et al., 2003). Sharks are further divided into two major lineages: Galea, consisting of Orectolobiformes, Heterodontiformes, Lamniformes, and Carcharhiniformes; and Squalea, consisting of Hexanchiformes, Echinorhiniformes, Squaliformes, Squatiniformes, and Pristiophoriformes (Nelson, 2006).

RESULTS

Character Combinations

Seventeen character traits are present among the 50 genera—seven different slit length morphologies (Fig. 3), five different slit spacing morphologies (Fig. 4), and five different numbers of slits over the fin (Fig. 5)—and are summarized in Table 1.
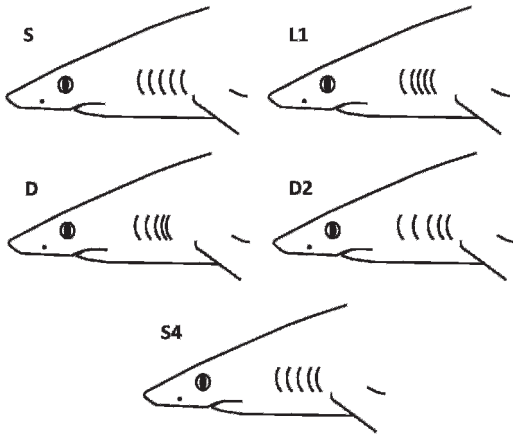
Figure 4. Schematics illustrating relative inter-gill slit distance character states. S, similar distance between all slit pairs; L1, distance between the first pair of slits is longer than the other pairs (1>234); D, distance increases among slit pairs in the anterior to posterior direction in at least three groupings (1>3>4); D2, distance increases among slit pairs in the anterior to posterior direction in at least two groupings (12>4); S4, distance between the last pair of slits is shorter than the other pairs (123>4).
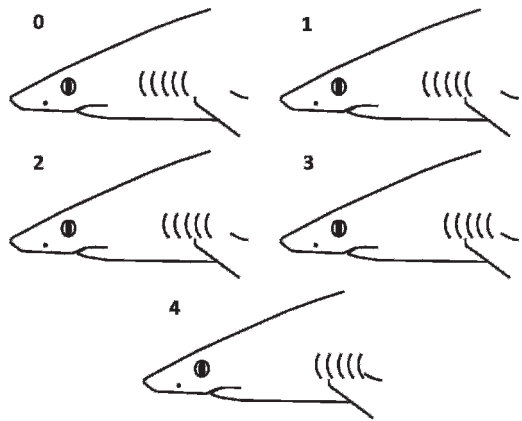

Figure 5. Schematics illustrating the number of gill slits positioned completely over the anterior border of the pectoral fin. 0, No slits over the fin; 1, one slit over the fin; 2, two slits over the fin; 3, three slits over the fin; 4, four slits over the fin.

Morphological measurements are listed in Appendices 1 and 2, and detailed statistics are presented in Appendices 3 and 4, with a complete list of specimens in the Supplementary Appendix. Characters were mapped onto the composite cladogram resolved to genera representing the samples (Fig. 6).

Few lamniform and hexanchiform species were available because of their large size; therefore, to keep these important orders in the analyses, some individuals from different genera were combined for the gill slit length and spacing statistical analyses, but only when they had the same characteristics. We only had one specimen of the lamniform species *Cetorhinus*, *Carcharodon*, *Mitsukurina*, and *Odontaspis* and only one specimen of the hexanchiform species *Heptranchias*. This was only done when the characteristics were the same to determine relative slit length and spacing differences within a genus: 5 of 126 analyses of variance included more than one genus. The genera were combined and a new combined analysis of variance and multiple comparison test was run: *Alopias* and *Odontaspis* were combined, and *Mitsukurina, Cetorhi-*

nus, Isurus, and *Carcharodon* were combined for slit lengths; *Isurus* and *Mitsukurina* were combined, and *Odontaspis, Cetorhinus, and Carcharodon* were combined for slit spacing; and *Heptranchias* and *Notorynchus* were combined for slit length and spacing. These were also all juveniles; therefore, examination of adult specimens is needed to determine whether the traits observed for the juveniles are retained in adults. A qualitative search of sevengill shark images on the worldwide web is consistent with the results here.

When juvenile and adult specimens were sampled within a species, they were tested and listed separately when they differed. For example, when multiple comparison tests for the analysis of variance gave different results for adults than for juveniles, they were listed separately with an A or J in the Stage column of the table. If there were no differences in the multiple comparison tests, juveniles and adults were combined, and a new combined analysis of variance and multiple comparison test was run, with an AJ listed in the Stage column of the table. Adult characteristics were used in the phylogenetic analyses unless only juvenile data were available. No node changes resulted from including juvenile data except within Lamniformes, because all of those

Table 1. Summary gill slit characters by genus showing length-space combinations.

Order and Family	Genus	Abbrev.	C No.	Length Stage[a]	Length State[b]	Space Stage[a]	Space State[b]	No. of Slits over Fin
Heterodontiformes								
Heterodontidae	*Heterodontus*	Heter	7	A	D	AJ	S	3
Heterodontidae	*Heterodontus*	Heter	7	J	D	AJ	S	3
Orectolobiformes								
Hemiscyllidae	*Chiloscyllium*	Chilo	4	AJ	S	A	D2	3
Hemiscyllidae	*Chiloscyllium*	Chilo	2	AJ	S	J	S4	2–3
Orectolobidae	*Orectolobus*	Orect	1	A	S	A	S	4
Stegostomatidae	*Stegostoma*	Steg	3	J	S	J	D	3
Carcharhiniformes								
Carcharhinidae	*Carcharhinus*	Car	6	AJ	S5	A	S	1
Carcharhinidae	*Carcharhinus*	Car	16	AJ	S5	J	D2	1–2
Carcharhinidae	*Galeocerdo*	Galeo	1	A	S	A	S	2
Carcharhinidae	*Loxodon*	Lox	1	J	S	J	S	1–2.5
Carcharhinidae	*Rhizoprionodon*	Rhiz	1	A	S	AJ	S	1
Carcharhinidae	*Rhizoprionodon*	Rhiz	8	J	D2	AJ	S	0–2
Carcharhinidae	*Scoliodon*	Scol	1	A	S	A	S	1
Hemigaleidae	*Hemipristis*	Hprist	1	A	S	A	S	1
Proscyllidae	*Eridacnis*	Erid	12	A	D2	A	L1	1
Proscyllidae	*Proscyllium*	Pro	5	A	L1	A	S	1
Scyliorhinidae	*Apristurus*	Apris	11	A	D2	A	D2	0–1
Scyliorhinidae	*Atelomycterus*	Atel	16	A	S5	A	D2	1
Scyliorhinidae	*Cephaloscyllium*	Ceph	1	AJ	S	AJ	S	2
Scyliorhinidae	*Galeus*	Gal	14	A	S5	A	S4	1, 2, or 4
Scyliorhinidae	*Haelaelurus*	Hael	7	A	D	A	S	1 or 3
Scyliorhinidae	*Haploblepharus*	Hap	8	A	D2	A	S	2
Scyliorhinidae	*Parmaturus*	Parm	1	A	S	A	S	2
Scyliorhinidae	*Poroderma*	Por	1	A	S	A	S	2
Scyliorhinidae	*Schroedericthyes*	Schroed	11	A	D2	A	D2	1–2
Scyliorhinidae	*Scyliorhinus*	Scyl	10	A	D	A	D2	1
Scyliorhinidae	*Scyliorhinus*	Scyl	7	J	D	J	S	1
Sphyrnidae	*Sphyrna*	Sphy	11	J	D2	J	D2	0–1
Triakidae	*Galeorhinus*	Grhin	1	J	S	J	S	1
Triakidae	*Hemitriakis*	Htri	1	J	S	J	S	1
Triakidae	*Mustelus*	Mus	15	A	S5	AJ	L1	1
Triakidae	*Mustelus*	Mus	12	J	D2	AJ	L1	1
Triakidae	*Triakis*	Tria	11	J	D2	J	D2	1–2
Lamniformes								
Mitsukurinidae	*Mitsukurina*	Mit	1	J	S	J	S	0
Odontaspididae	*Odontaspis*	Odon	2	J	S	J	S4	0
Alopidae	*Alopias*	Alo	4	J	S	J	D2	2
Cetorhinidae	*Cetorhinus*	Cetor	2	J	L1	J	S4	0
Lamnidae	*Isurus*	Isur	1	J	L1	J	S	0
Lamnidae	*Carcharodon*	Cchar	2	J	L1	J	S4	0
Hexanchiformes								
Chlamydoselachidae	*Chlamydoselachus*	Chlam	1	A	S	A	S	0
Hexanchidae	*Hexanchus*	Hex	1	J	S	J	S	0
Hexanchidae	*Heptranchias*	Hept	10	J	D	J	D2	0
Hexanchidae	*Notorynchus*	Noto	10	J	D	J	D2	0
Squaliformes								
Squalidae	*Squalus*	Squa	17	A	L5	AJ	D	0
Squalidae	*Squalus*	Squa	3	J	S	AJ	D	0
Centrophoridae	*Centrophorus*	Cphor	13	A	I2	A	S	0

TABLE 1.   CONTINUED.

Order and Family	Genus	Abbrev.	C No.	Length Stage[a]	Length State[b]	Space Stage[a]	Space State[b]	No. of Slits over Fin
Centrophoridae	*Centrophorus*	Cphor	4	J	S	J	D2	0
Centrophoridae	*Deania*	Dea	1	J	S	J	S	0
Etmopteridae	*Etmopterus*	Etmo	11	A	D2	A	D2	0
Etmopteridae	*Etmopterus*	Etmo	1	J	S	J	S	0
Somniosidae	*Centroscymnus*	Cscym	1	J	S	J	S	0
Oxynotidae	*Oxynotus*	Oxyn	1	A	S	A	S	0
Dalatiidae	*Centroscyllium*	Cscyl	2	AJ	S	A	S4	0
Dalatiidae	*Centroscyllium*	Cscyl	1	AJ	S	J	S	0
Dalatiidae	*Dalatias*	Dal	2	J	S	A	S4	0
Dalatiidae	*Euprotomicrus*	Eupro	1	A	S	A	S	0
Dalatiidae	*Isistius*	Isis	1	A	S	A	S	0
Dalatiidae	*Scymnodon*	Scym	11	AJ	D2	AJ	D2	0
Pristiophoriformes								
Pristiophoridae	*Pristiophorus*	Prist	1	A	S	A	S	0
Squatiniformes								
Squatinidae	*Squatina*	Squat	13	J	I2	J	S4	0

[a] Juveniles (J) and adults (A) are listed separately when they differ.
[b] S, similar lengths among all slits; D, decreasing anterior to posterior with at least three different groups; D2, decreasing anterior to posterior in only two different groups; L, longer followed by slit number that is longer than all others; I2, increasing anterior to posterior in only two groups; S, shorter followed by slit number that is shorter than all others.

specimens were juvenile, and at the base of the two sevengill genera, because those were also juvenile. This grouping may introduce potential error; however, the benefits of a more complete analysis and having a baseline for further studies is key here. Including genera that only contained juvenile data increased the number of genera in the study by 40% (indicated by a J after the genera in Fig. 6); therefore, they were retained in favor of a more complete phylogenetic analysis and baseline, noting that in some cases there might be ontogenetic differences that may not represent the adult condition. Of the 50 genera analyzed, 19 contained only adult specimens, 12 contained adult and juvenile specimens, and 19 contained only juvenile specimens. Ontogenetic changes occurred in 10 of the 12 genera that had adult and juvenile specimens that resulted in assignment to a different category (such as D2 to D or S to

S5): 42% changed in slit length (five genera), 50% changed in slit spacing (six genera), and 25% (three genera) changed in number of slits over the fin.

Relative Gill Slit Length

Seven character states of relative gill slit length are present among the genera represented (D, D2, I, L1, L5, S, S4) (Fig. 3; Table 1). A total of 62 analyses of variance were run. The most common character state is to have slits of similar length (S). The most common character state change is to have slits that decrease in length from anterior to posterior. Increasing length occurs where at least three groups of different consecutive slit lengths exist (I). Decreasing length occurs in several states, in which at least three groups of different consecutive slit lengths exist (D) and two different slit lengths exist that may or may not be in consecutive groups (D2). Two

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Figure 6.   Gill slit characters and ventilation mode mapped onto a selachian phylogeny. Numbers within the boxes correspond to the character state. D, At least three groups of different decreasing consecutive slit lengths/spaces exist; D2, two different decreasing slit lengths/spaces exist that may or may not be in consecutive groups; I2, two different increasing slit lengths exist that
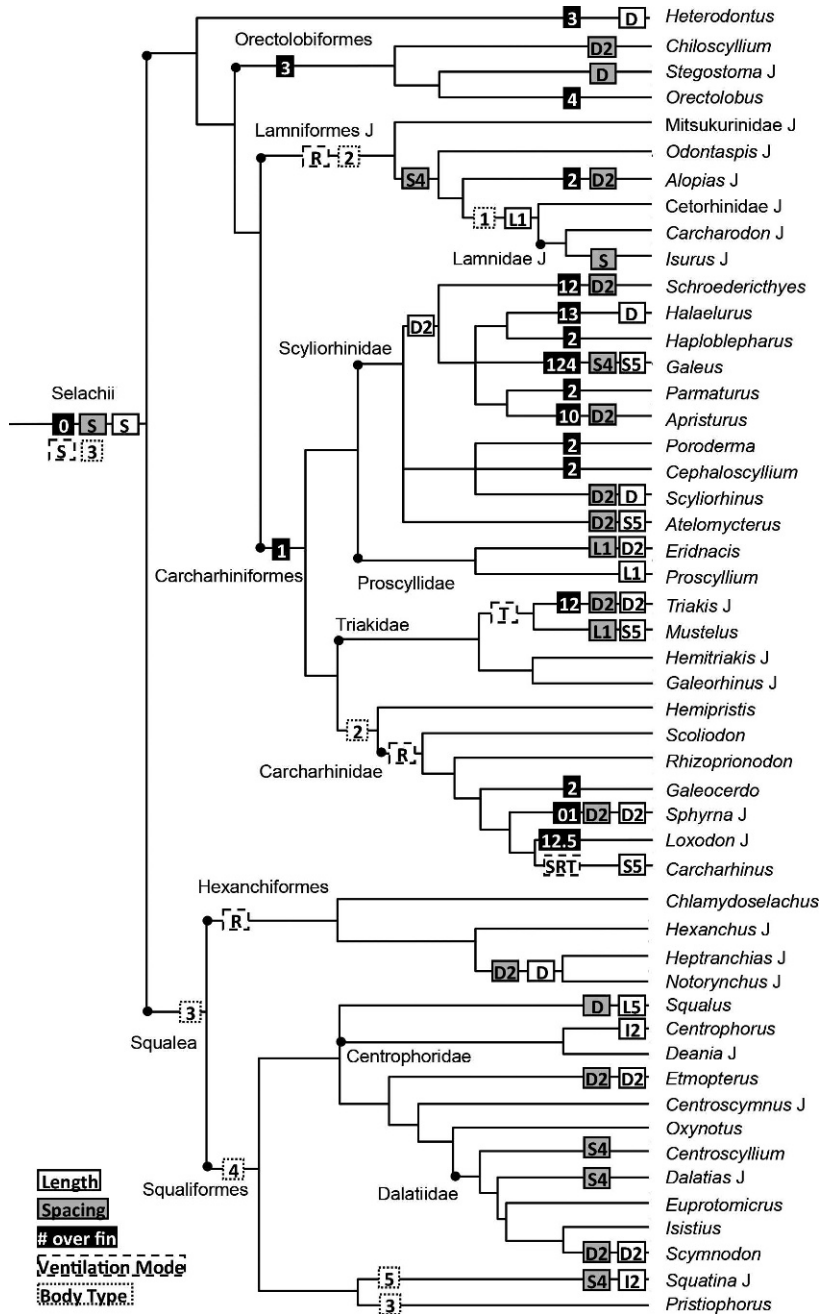
may or may not be in consecutive groups; L1, longer first slit or wider first spacing; S, similar length or spacing; S5, shorter last slit or narrower last spacing. Numbers over the fin indicate all combinations found in the genera (01 means 0 and 1 slit over the fin, 12.5 means 1 and 2.5 slits over the fin). S, R, or T in dashed box indicates suction, ram, or transition ventilation. Numbers in dotted box indicate body type: 1, macropelagic; 2, littoral; 3, benthic; 4, bathic/micropelagic. Branches do not indicate divergence time. A "J" after a genus indicates results are based on juvenile specimens and may not represent the adult condition. (Cladogram compiled after Naylor, 1992; Shirai, 1996; Goto 2001; Douady et al., 2003; Human et al., 2006; Lopez et al., 2006).

states that could lead to decreasing slit lengths, as in D and D2, occur where the first slit is longer than the rest (L1) or where the last slit is shorter than the rest (S5).

Relative lengths among the slits are similar (S) for 26 of the 50 genera (Table 1; Fig. 3, S). The gill slits increase in length from anterior to posterior, with two different groupings or lengths among the slits in two genera: 123<45 in adult *Centrophorus*; 1<45 in *Squatina* (Appendices 1 and 3; Fig. 3, I2). Note that for ease of determining where the differences are, when a character number is not listed, it is not different from the states on either side of the sign. For example, in the 1<45 example above, gill slits 2 and 3 are not different from slit 1, nor are they different from slits 4 and 5, but gill slit 1 is shorter than slits 4 and 5. Four genera show decreasing length from anterior to posterior, with at least three different groupings or lengths among the slits (1>23>45 in adult and 12>3>45 in juvenile *Heterodontus*; 1>23>5 in *Halaelurus*; 1>2>4>5 in adult and 12>34>5 in juvenile *Scyliorhinus*; 1>23>34>45>56 >67 in *Heptranchias* (Appendix 1; Fig. 3, D). Nine genera show the same decreasing length but with only two different length groupings among the slits: 1>2 in adult *Etmopterus*; 1>45 in *Haploblepharus*; 1>5 in *Eridacnis* and *Scymnodon*; 12>5 in *Schroedericthyes*; 123>5 in *Apristurus* and *Sphyrna*; and 3>5 in juvenile *Rhizoprionodon* and *Mustelus* (Appendix 1; Fig. 3, D2). In two genera, slit 1 or 5 is relatively longer than all of the others, which have similar lengths: 1>2345 in *Proscyllium*; 1234<5 in adult *Squalus* (Appendix 1; Fig. 3, L1, L5). In three genera, the first slit is longer than slit 3: 1>3 in *Cetorhinus*, *Carcharodon,* and *Isurus* (Appendix 1; Fig. 3, L1). In four genera, slit 5 is relatively shorter than all of the others, which have similar lengths: 1234>5 in *Carcharhinus*, *Atelomycterus*, *Galeus,* and adult *Triakis* (Appendix 1; Fig. 3, S5).

Gill slit variables mapped onto the compiled cladogram revealed clear evolutionary relationships in most of the characters in the taxa analyzed here. In the most parsimonious scenario, the basal state for modern sharks is slits of similar length with 18 character state changes among the taxa—most at the branch tips, with only three at shallow nodes (Fig. 5, white squares). Galea retained the basal character state (S), with the most common state change to slits of decreasing length (D) in *Heterodontus* and some carcharhiniform genera, whereas slits of similar length (S) are retained in orectolobiform and basal lamniform genera, and a longer first slit (L1) evolved in derived lamniform genera. Adult-derived lamniform specimens need to be examined to determine whether these juvenile states are retained in adults. Eight of the 12 scyliorhinid genera evolved decreasing slits (D, D2), whereas two others evolved a shorter fifth slit (S5). The same two character states evolved in triakid and carcharhinid genera: D2 in *Triakis* and S5 in *Mustelus*, and D2 in *Sphyrna* and S5 in *Carcharhinus*. Two character states evolved in proscyllid genera, D2 and L1.

The basal state for the squalean lineage is also slits of similar length (S). The basal state was retained in frilled and sixgill sharks (S), *Chlamydoselachus* and *Hexanchus*, the two basal genera in Hexanchiformes, whereas sevengills evolved slits of decreasing length (D), *Heptranchias* and *Notorynchus*. Adult sevengill specimens need to be examined to be sure the juvenile trait is retained in adults. However, a search of sevengill shark images on the worldwide web (there are only two species) indicates that they all have qualitatively decreasing slit lengths. Squalid genera evolved a longer fifth slit (L5), *Etmopterus* and *Scymnodon* evolved decreasing slit lengths (D2), and *Centrophorus* evolved increasing slit lengths (I2). *Squatina* evolved increasing gill slit lengths (I2), whereas *Pristiophorus* retained similar lengths (S).

Relative Inter-Gill Slit Distances

Five character states of relative inter-gill slit distances are exhibited among the genera represented (D, D2, L1, S, S4)

(Fig. 4; Table 1). A total of 64 analyses of variance and multiple comparison tests were run. The most common character state for slit spacing is to have similar spacing between all slits (S). Again, the most common character state change is to have slit spacing that decreases in distance (length between adjacent slits) from anterior to posterior. Decreasing spacing occurs in the same states as that for length. Note that the last space in species with five gill slits is the fourth one (sixgill and sevengill species have similar spacing).

Inter-gill slit distances between all adjacent pairs of gill slits are similar to one another in 29 of the 50 genera (Table 1; Fig. 4, S). Two genera show decreasing distance between slits from anterior to posterior, with at least three different groupings or distances among the slits: 1>3>4 in *Stegostoma* and 12>23>34 in *Squalus* (Appendices 2 and 4; Fig. 4, D). Fifteen genera show decreasing distance between slits from anterior to posterior, but with only two different distance groupings among the slits: 1>34 in *Apristurus*, juvenile *Centrophorus*, and *Etmopterus*; 1>4 in *Atelomycterus*, *Schroedericthyes*, adult *Scyliorhinus*, and *Scymnodon*; 1>56 in *Heptranchias* and *Notorynchus*; 12>4 in adult *Chiloscyllium*, *Carcharhinus*, *Galeus*, *Sphyrna*, *Triakis*, and *Alopias* (Appendix 2; Fig. 4, D2). In two genera, the distance between the first pair of slits is relatively longer than all of the others, which have similar lengths; 1>234 in *Eridacnis* and *Mustelus* (Appendix 2; Fig. 4, L1). In seven genera, the distance between the last pair of slits is relatively shorter than all of the others, which have similar distances; 123>4 in *Odontaspis*, *Cetorhinus*, *Carcharodon,* adult *Centroscyllium*, *Dalatias*, *Squatina*, and juvenile *Chiloscyllium* (Appendix 2; Fig. 4, S4).

In the most parsimonious scenario, the basal state for modern sharks is slits of similar spacing (S) with 21 character state changes among the taxa, all at the branch tips except for two at shallow nodes (Fig. 6, gray squares). The basal state for the galean lineage is to have similar spacing (S) and is retained in *Heterodontus*. Character state changes evolved in two basal orectolobiform genera, *Chiloscyllium* (D2) and *Stegostoma* (D), whereas the basal state was retained in the derived orectolobid genus *Orectolobus* (S). A shorter last space (S4) evolved early in Lamniformes, after *Mitsukurina*, with further space differences evolving in *Alopias* (D2) and a reversal back to similar spacing in *Isurus* (S). However, adult lamniform specimens need to be examined to test whether these states hold for adults as well. Carcharhiniform and scyliorhinid genera again have the greatest number of changes in spacing state, with similar spacing (S) being the basal state for both, as was previously found for Scyliorhinidae (Compagno, 1988). Decreasing slit spacing (D2) evolved in four scyliorhinid groups, whereas a shorter last spacing (S4) evolved in only one. A longer first slit space (L1) evolved in one proscyllid group. Again, slit spacing state changes evolved in triakid genera: D2 in *Triakis* and L1 in *Mustelus*. Finally, only one carcharhiniform group evolved a slit space change, *Sphyrna* (D2).

The basal state for the squalean lineages is to have similar spacing (S) among the slits. One character state change evolved in the more derived hexanchiform genera, *Heptranchias* and *Notorynchus,* to decreasing slit spacing (D2). Again, adult sevengill specimens need to be examined to be sure the traits hold for adults. However, it appears that the last one or two slit spaces are shorter than more anterior ones in images of adult sevengill sharks on the worldwide web. The basal state was retained in squaliform genera (S); however, three genera evolved decreasing slit spacing: *Squalus* (D), *Etmopterus* (D2), and *Scymnodon* (D2). Three genera evolved a shorter last space (S4): *Centrosyllium, Dalatias*, and *Squatina*.

Number of Slits over the Pectoral Fin

Five character states of gill slit position over the fin exist among the 50 genera (Table 1;

Fig. 5). The most common character state for number of slits positioned over the pectoral fin is none (0). Thirty-three genera have no slits over the fin: some *Sphyrna*, some adult *Apristurus*, some juvenile *Rhizoprionodon*, all Lamniformes except *Alopias*, and all Squalea (Table 1; Fig. 5-0). Twenty-six genera have one slit over the fin (1): *Scoliodon*, *Hemipristis*, *Eridacnis*, *Proscyllium*, some *Apristurus*, *Atelomycterus*, some *Galeus*, some *Halaelurus*, some *Schroedericthyes*, *Scyliorhinus*, some *Sphyrna*, some *Triakis*, *Galeorhinus*, *Hemitriakis*, *Mustelus*, and all adult and some juvenile *Carcharhinus* and *Rhizoprionodon* (Table 1; Fig. 5-1). Seventeen genera have two slits over the fin (2): *Galeocerdo*, some *Galeus*, *Cephaloscyllium*, *Haploblepharus*, *Parmaturus*, *Poroderma*, some *Schroedericthyes*, some *Triakis*, *Alopias*, some juvenile *Chiloscyllium*, and some juvenile *Carcharhinus* (Table 1; Fig. 5-2). Five genera have three slits over the fin: *Heterodontus*, *Stegostoma*, all adult and some juvenile *Chiloscyllium*, and some *Halaelurus* (Table 1; Fig. 5-3). Finally, two genera have four sits over the fin: *Orectolobus* and some *Galea* (Table 1; Fig. 5-4). Eight genera contain species with more than one state of slit number of the pectoral fins: 1-2 in *Carcharhinus*; 1-2.5 in *Loxodon*; 0-2 in juvenile *Rhizoprionodon*, 0-1 in *Apristurus*; 1, 2, or 4 in *Galeus*; 1 or 3 in *Halaelurus*; 1-2 in *Schroedericthyes*, 0-1 in *Sphyrna*, and 1-2 in *Triakis*. Thus, two different character states of slit position over the fin exist within five genera, and three different character states exist in three other genera.

In the most parsimonious scenario, the basal state for modern sharks is to have no slits over the pectoral fin with 25 character state changes among the taxa, all at the branch tips except for two at nodes (Fig. 6, black squares). The most parsimonious hypothesis for the basal state in the extant galean lineage is to have no slits positioned over the pectoral fin (0); however, three slits over the fin (3) cannot be ruled out. The basal state for number of slits over the fin is unresolved for the galean lineage: the basal state may be three, as in the two basal groups (3) (Heterodontiformes and Orectolobiformes), with zero (0) evolving in Lamniformes and one (1) in Carcharhiniformes, or the basal state may be zero (0), as retained in Lamniformes and Squalea. It is more parsimonious that the basal state is zero, as in Squalea, and that Carcharhiniformes evolved one slit over the fin while Heterodontiformes and Orectolobiformes independently evolved three slits over the fin than for Lamniformes and Carcharhiniformes to lose two or three slits over the fin.

Heterodontus and *Chiloscyllium* have three slits over the fin (3), whereas *Orectolobus* evolved four (4). The basal state for Lamniformes is to have no slits over the pectoral fin (0); however, *Alopias* evolved two slits over the fin (2). Adult lamniform specimens need to be examined to determine whether the trait is retained in adults. The basal state for Carcharhiniformes and Scyliorhinidae (Compagno, 1988) is to have one slit over the pectoral fin (1). The number of slits over the fin varies greatly among genera within Carcharhiniformes. The most common state change is to have two slits over the fins (2), which evolved in five scyliorhinid, one triakid, and three carcharhinid genera. Four slits over the pectoral fin (4) evolved in one scyliorhinid genus, and no slits over the pectoral fins (0) evolved in two carcharhiniform genera. All of the squalean genera examined have no slits positioned over the pectoral fin (0).

Evolutionary Relationships in Gill Slit Morphology of Extinct and Extant Groups

Fossil chondrichthyan data may clarify the ancestral state of number of gill slits over the fin. Cladoselachida is the sister group to Elasmobranchii, with Xenacanthida a basal Elasmobranchii (Maisey, 1982; Wilga, 2002). Ctenacanthiformes is the sister group to Euselachii with Hybodontiformes the basal euselachian and Synechodontiformes the sister group to neoselachians (Galea and Squalea) (Schaeffer and Williams, 1977; Maisey, 1982;

	PC1	PC2	PC3	PC4
Adult variables				
Length	0.5444	0.4088	−0.5137	0.5221
Space	0.1508	0.8109	0.4161	−0.3828
Slits	0.5633	−0.3035	0.6930	0.3322
Body type	−0.6030	0.2884	0.2877	0.6859
Juvenile variables				
Length	0.5772	−0.0272	−0.3552	0.7347
Space	0.3804	0.8533	0.3417	−0.1021
Slits	0.4551	−0.5002	0.7364	−0.1998
Body type	−0.5612	0.1448	0.4634	0.6703

P, principal component.

Grogan and Lund, 2004). Gill slit tissue does not preserve well if at all during the fossilization process; however, inferences can be made about the position of the gill arches to the pectoral girdle in extinct genera. According to illustrations of fossil reconstructions, none of the gill arches were positioned over the pectoral girdle in *Cladoselache* spp. (Cladoselachida), *Triodus* spp. (Xenacanthida), *Hybodus* spp. (Hybodontoidea), and *Paleospinax* (Synechodontiformes) (Zangerl, 1981; Cappetta, 1987). Thus, it is likely that the gill slits were also not positioned over the fin in these groups, and the basal state for the number of slits over the fin in extant neoselachians is none (0). The number of branchial arches over the pectoral girdle agrees with the number of gill slits over the pectoral fin in intact extant *Chiloscyllium*, *Squalus*, *Isurus*, and *Pristiophorus* (Hamlett, 1999; personal observation), indicating that this assessment may be reliable. If so, then the basal state for number of slits over the fin in Galea is none (0).

Principal Components Analysis

The principal components analysis (PCA) showed that correlations among the variables were largely similar between juveniles and adults; however some ontogenetic differences were revealed. The first two principal components (PCs) accounted for 75% of the variation in adults and 70% in juveniles (Table 2). Three variables load similarly on PC1 in adults and juveniles, with number of slits over the fin and slit length loading highly positive and body type loading highly negative (Fig. 7). On PC2, slit spacing loads highly positive in adults and juveniles, whereas number of slits over the fin loads negatively in juveniles.

DISCUSSION

Phylogenetic Relationships in Gill Slit Morphology

The basal state for Selachii, as well as Galea and Squalea, is to have similar slit lengths and spacing and no slits over the fin. Similar character trait changes occurred broadly across the clades, with some differences among shark orders. All the shark orders except Lamniformes had genera that evolved decreasing consecutive slit lengths (D). Three lamniform genera and one carcharhiniform genus evolved a longer first slit (L1). Eight carcharhiniform and two squaliform genera evolved some shorter nonconsecutive gill slits (D2). A shorter last (S5) slit only evolved in four carcharhiniform genera, whereas a longer fifth slit (L5) and increasing slit lengths (I2) only evolved in one and two squaliform genera, respectively. More broadly, only galean genera evolved L1 (length and space) and S5 (length) traits, whereas only squalean genera evolved I2 and L5 (both length) traits, with both clades evolving D and D2 (length and space) traits.
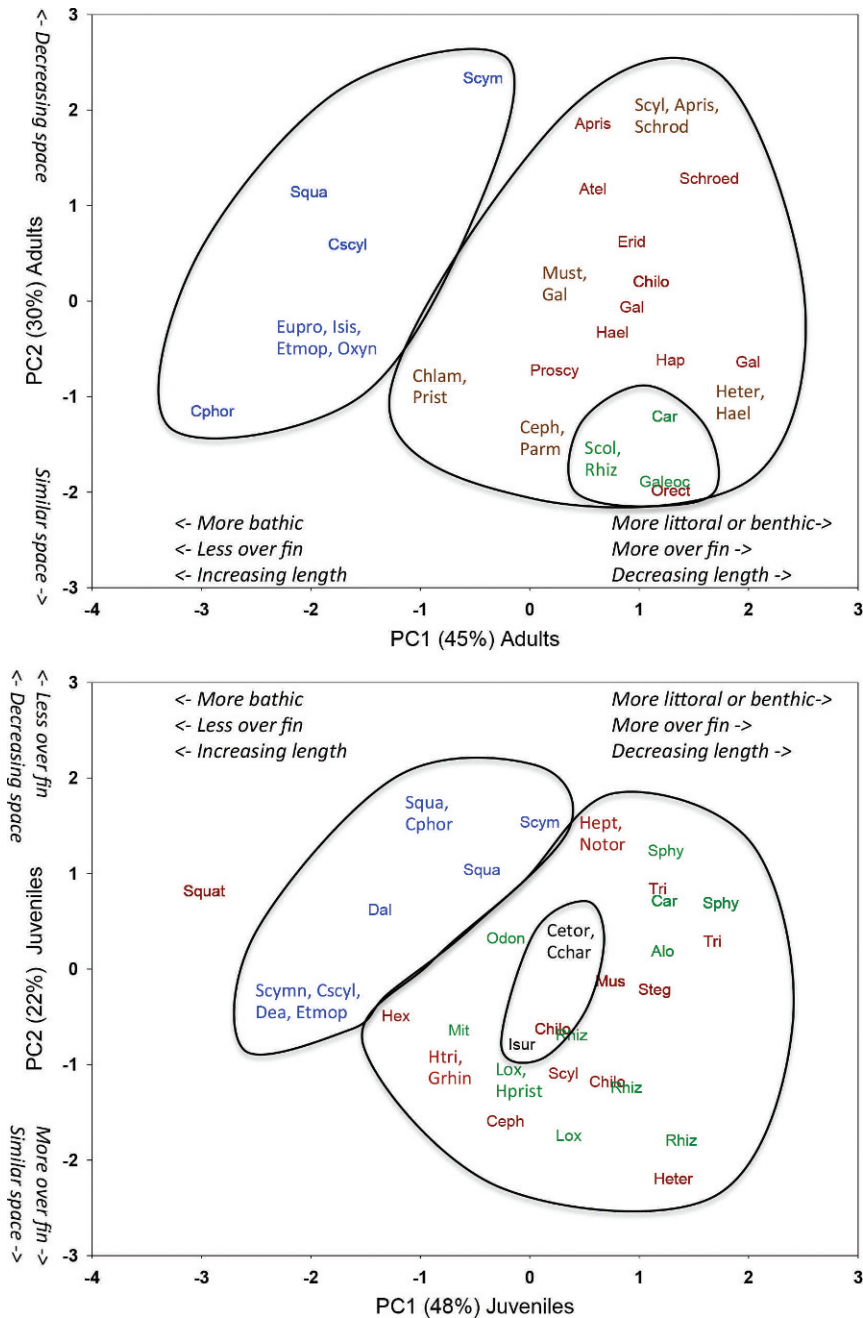
Figure 7.   PC1 and PC2 of gill slit morphology and body type in adults (top) and juveniles (bottom). When the values for length, space, slit, and body type differ among species, the position is indicated separately using the genus name for clarity; thus, multiple genera may appear more than once in the PCA if different combinations of the four values exist among species within a genus. See Table 2 for abbreviations. Colors indicate body type: black, 1 macropelagic; green, 2 littoral; brown, 3 benthic/bathic; blue, 4 micropelagic, squaliform. Overlapping species are expanded as a list with commas centered over the PCA position. Circles encompass body types.

Similarly, all of the shark orders had genera that evolved some nonconsecutive smaller slit spaces (D2). One orectolobiform, one carcharhiniform, three lamniform, and three squaliform genera evolved a smaller last space (S4), whereas only two carcharhiniform genera evolved a longer first space (L1). One orectolobiform, two squaliform, and two hexanchiform genera evolved decreasing consecutive spacing (D, D2). The only reversal found was back to similar slit spacing (S) in juvenile *Isurus* (Lamniformes).

Other slit trait changes may be worth noting. Heterodontiform and orectolobiform genera have three or four slits over the pectoral fin and one lamniform genus evolved two slits, while the trait changed numerous times in carcharhiniform genera to all states (0, 2, 3, 4). It also appears that the addition of one more arch retains similar slit lengths and spacing (*Chlamydoselachus* and *Hexanchus*), whereas the addition of two more gill arches results in decreasing slit lengths and spacing (*Heptranchias* and *Notorynchus*).

Many of the character state changes in slit morphology evolved within two families in the order Carcharhiniformes: 10 genera in Scyliorhinidae and four genera in Carcharhinidae. The most common change in slit length was to decreasing lengths (D2, D) in 10 genera, with a shorter fifth slit (S5) in three genera and a longer first slit (L1) in one genus. Decreasing slit spacing was also the most common space change (D2) in six genera, whereas a longer first slit space (L1) evolved in two genera, and a shorter last space (S5) evolved in one genus. The most common state change in number of slits over the fin is to evolve an additional slit over the fin (2), which occurred in eight genera, with an additional one or two more slits (3-4) over the pectoral fin evolving in two other scyliorhinid genera.

Interrelationships between Gill Slit Morphology and Body Type

Exploring the interrelationships among gill slit morphology and body type in a PCA reveals similar correlations between adults and juveniles. The genera fall out into three groups in PCA morphospace that distinguishes some of the body types in adults (circled in Fig. 7). Micropelagic/bathic body types are on the left upper side (blue), which also only contains squalean genera. Littoral body types occupy a small area in the lower right side (green) that is contained within the benthic body types (brown) that encompasses all of the right side and some of the lower left side. A similar pattern exists for juveniles, except that macropelagic body types (black) are clustered within the broadly mixed littoral and benthic body type space (circled in Fig. 7).

The two major sharks groups are separated by the first two axes of the PCA. Galean sharks with littoral and benthic body types (green and brown, respectively) occupy the positive side of PC1. These genera tend have more slits over the fin and slits of similar or decreasing length. Squalean genera are unique in having a bathic or micropelagic body type (blue), and occupy the negative side of PC1. All squalean genera have no slits over the fin and, unlike galean genera, include increasing slit lengths as well as similar and decreasing slit lengths. *Chlamydoselachus*, the most basal squalean, and *Pristiophorus*, the most derived squalean, have the benthic body type (brown) and load slightly negative between the rest of the squalean and galean genera. The macropelagic genera load around the origin in the juvenile PCA; adult specimens need to be examined to see where they fall out on the adult plot. The number of slits over the fin is slightly less positive for juveniles, indicating that they tend to have fewer slits over the fin than adults. The genera that load most negatively are the only two to have increasing slit lengths (*Squatina* and *Centrophorus*).

Slit spacing was the most informative characteristic on PC2 for adults and juveniles, with number of slits over the fin also a useful characteristic for juveniles. Adult and juvenile genera with similar slit spacing load on the negative side of PC2, whereas genera

with decreasing slit spacing load on the positive side. In juveniles, the number of slits over the fin load more negatively than adults, indicating that juveniles tend to have an inverse relationship between spacing (more decreasing) and number of slits (fewer) over the fin.

Ontogenetic Changes in Gill Slit Morphology and Body Type

Ontogenetic changes in gill slit morphology were discovered in the 12 genera in which data from juvenile and adult specimens were collected. More ontogenetic changes occurred in gill slit length and spacing than number of slits over the fin. There were no ontogenetic differences in gill slit length in five of those genera: *Chiloscyllium*, *Carcharhinus*, *Cephaloscyllium*, *Centroscyllium*, and *Scymnodon*. In the remaining genera with ontogenetic changes in gill slit length, 71% of the changes lead to increasing differences among the slits (S to I2, D2, or L5 in *Centrophorus*, *Etmopterus*, and *Squalus*), whereas the rest of the changes are either to become more similar (D2 to S, *Rhizopriono-don*), to have fewer differences (D2 to S5 in *Mustelus*), or to result in a different combination of changes under the same character state (remains D, as in *Scyliorhinus*, *Heterodontus*). In contrast, ontogenetic changes were equally split between more and fewer differences in gill slit spacing: more in *Scyliorhinus*, *Centroscyllium*, and *Etmopterus* and fewer in *Chiloscyllium*, *Centrophorus*, and *Carcharhinus*. There were no ontogenetic differences in gill slit spacing in six genera: *Heterodontus*, *Rhizoprionodon*, *Cephaloscyllium*, *Mustelus*, *Scymnodon*, and *Squalus*. Only three of the 12 genera had ontogenetic differences in the number of slits over the fin: *Chiloscyllium* have more over the fin as adults, and *Carcharhinus* and *Rhizoprionodon* have fewer over the fin as adults. *Scyliorhinus* and *Etmopterus* had the most ontogenetic changes (length and spacing) of the 12 genera.

These ontogenetic changes are evident in the PCA plot of PC1 and PC2. Slit length changes more often to increasingly different (five genera) than to increasingly similar (two genera), with no differences also occurring (five genera) from juveniles to adults, and this is shown by the slightly less positive PC1 value in adults. Slit spacing has an equal tendency to become more similar (three genera) or more different (three genera), with an equal number having no changes (six genera) from juveniles to adults; thus, spacing moves closer to the origin in adults on PC1. The number of slits over the fin remains similar in most (nine genera) but has changed to more over the fin (one genus) or fewer over the fin (two genera) in some cases, with a slightly more positive number on PC1.

The relationship between body type and gill slit morphology is less distinct in juveniles compared with adults and is likely not only due to ontogenetic differences but also to habitat differences. Pelagic, littoral, and benthic genera overlap in juveniles, in contrast to littoral genera overlapping with only a few benthic groups in adult morphospace. Some shark species have pupping grounds that are in different habitats than adults, where newborns may reside for several years before moving on to the adult habitat (Heupel et al., 2007). These habitat differences may be related to changes in body type as a result of ontogenetic changes in morphology.

Ecomorphological Relationships of Gill Slit Morphology, Body Type, and Ventilation Mode

Exploring the ecomorphological relationships between gill slit morphology and ventilation mode in modern shark genera reveals character complexes that are common to the four simplified body types (Thomson and Simanek, 1977; Compagno, 1990), although exceptions may well occur. Sharks of body type 1 in this study are macropelagic ecomorphotypes and comprise *Cetorhinus*, *Carcharodon*, and *Isurus*
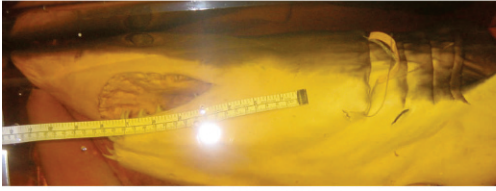
A) Body type 1 - Macropelagic Ecomorphotypes
<u>Taxa</u>: Cetorhinidae and Lamnidae
<u>Slit length</u>: Longer first
<u>Slit space</u>: 66% similar, 33% shorter last
<u>Slits over fin</u>: 0
<u>Ventilation</u>: ram

B) Body type 2 - Littoral Ecomorphotypes
<u>Taxa</u>: Mitsukurinidae, Odontaspididae, Alopidae,
Carcharhinidae
<u>Slit length</u>: 69% decrease, 31% similar
<u>Slit space</u>: 54% decrease, 46% similar
<u>Slits over fin</u>: 74% 1, 14% 2, 11% 0
<u>Ventilation</u>: 80% ram, 20% transition

C) Body type 3 – Benthic Ecomorphotypes
<u>Taxa</u>: Heterodontiformes, Orectolobiformes,
Carcharhiniformes except Carcharhinidae,
Hexanchiformes and Pristiophoriformes.
<u>Slit length</u>: 69% decrease, 31% similar
<u>Slit space</u>: 66% decrease, 34% similar
<u>Slits over fin</u>: 52% 1, 19% 2, 18% 3, 8% 0, 3% 4
<u>Ventilation</u>: 69% suction, 27% transition, 4% ram

**D) Body type 4 – Bathic and Micropelagic
Ecomorphotypes**
<u>Taxa</u>: Squaliformes
<u>Slit length</u>: 69% similar, 24% increase, 7% decrease
<u>Slit space</u>: 56% similar, 44% decrease
<u>Slits over fin</u>: 0
<u>Ventilation</u>: suction

Figure 8.   The four body types by ecomorphotype are shown with a representative species: A) Macropelagic, B) Littoral, C) Benthic, and D) Bathic and Micropelagic with associated taxa, slit length, slit space, number of slits over the fin, and ventilation mode.

genera (Thomson and Simanek, 1977; Compagno, 1990) (Fig. 8A). These genera are obligate ram ventilators, wherein continual forward motion of the swimming fish drives fluid flow over the gill filaments, rather than movement of head and branchial muscles as in suction ventilators (Roberts, 1975; Compagno, 1990; Thomson and Simanek, 1990). Even though the head is cone shaped in this group (Thomson and Simanek, 1990), there must be some added energetic expense to counter the increased drag of swimming with the mouth and gill slits open. Macropelagic genera have lateral keels on narrow caudal peduncles and high aspect ratio tails, which are characteristic of fast-swimming marine vertebrates (Thomson and Simanek, 1990). The first gill slit is longer in the macropelagic genera examined, which may function to decrease drag and limit flow resistance at the remaining gill slits when swimming at high velocities, as these genera typically do. Indeed, a higher volume of flow rate exits the anteriormost gill slit in model fish with conical oral cavities (Cheer et al., 2001). Accordingly, flow volume is greater at the first internal gill slit of *Cetorhinus*, which has a conical pharynx (Sims and Quayle, 1998; Cheer et al., 2001). However, until flow velocity is measured in lamnid genera, it is unknown whether this is due to the filter

feeding habit of *Cetorhinus* or is truly a characteristic of macropelagic body types. Macropelagic genera, especially regional endotherms like *Carcharodon* and *Isurus*, typically have higher metabolic rates and gill filament surface area than other ram-ventilating and benthic-dwelling genera (Emery and Szczepanski, 1986; Graham et al., 1990).

Most macropelagic genera have similar slit spacing, with 33% having a shorter last space (*Cetorhinus* and *Carcharodon*). Similar spacing provides consistently spaced exit sites for water throughout the pharynx, which is likely favorable to fast swimmers. Shorter spacing for the last slit may be associated with filter feeding in *Cetorhinus*, but it is not clear how this is advantageous for the gouge-feeding mechanism of *Carcharodon*. The pectoral fins of macropelagic genera are more posteriorly placed relative to total body length than those of benthic genera (Thomson and Simanek, 1977); thus, having all of the gill slits anterior to the pectoral fin may simply be a consequence of pectoral fin placement that results in uniform exhalation flow around the body. Indeed, shorter oropharyngeal cavities generated more internal vorticity than longer cavities, with more particles exiting the mouth in a modeling study of ram filter feeding in fish (Paig-Tran et al., 2011). However, more posteriorly placed pectoral fins may also provide maximum space for gill filament volume and the increased oxygen consumption that fast swimming requires. Filtering efficiency increased with size of the gill slits in models of ram filter feeding, indicating that water volume and gas diffusion may also increase with gill volume (Paig-Tran et al., 2011).

Sharks of body type 2 in this study are littoral ecomorphotypes and contain genera in the Family Carcharhinidae, as well as the more basal lamniforms (*Mitsukurina, Alopias,* and *Carcharias*). (Thomson and Simanek, 1977) (Fig. 8B). Littoral ecomorphotypes live in a variety of habitats and are characterized by having a "generalized" body form with a flat head, small spiracles and gill openings, and moderately sized pectoral fins (Compagno, 1990). Littoral genera differ from macropelagic genera in the tendency to have gill slits and spacing that decrease in length, with one or two slits over the pectoral fin. The remaining genera have similar lengths and spacing and no slits over the fin. The predominant ventilatory mode is obligate ram, as it is in macropelagic genera, and thus most littoral genera must continuously swim to ventilate the gills (Thomson and Simanek, 1977; Compagno, 1984). However, some suction-ventilating littoral genera are capable of generating water flow over the gill filaments while resting on the substrate, and some may transition to ram when swimming (Compagno, 1984; Graham et al., 1990). Transition-ventilating littoral genera generally have one slit over the fin that is shorter than the rest. Perhaps the last slit functions as an exit valve to avoid excess pooling of water in the pharynx. The generally slower swimming speeds of littoral genera, compared with macropelagic genera, may reduce the induced drag that decreasing slit lengths and spacing may incur when swimming. It is not clear whether the additional flow over the pectoral fin from one or two gill slits would be substantial enough to help or hinder pectoral fin function hydrodynamically (Wilga and Lauder, 2000, 2001, 2004).

Benthic ecomorphotypes characterize body type 3 and comprise nearly half of the genera in this study in several orders: Hexanchiformes, Pristiophoriformes, Heterodontiformes, Orectolobiformes, and Carcharhiniformes except Carcharhinidae (Thomson and Simanek, 1977) (Fig. 8C). These genera spend much of their time resting on the substrate (Compagno, 1990) and differ from littoral body types in having more genera with decreased spacing, more slits over the fin (up to four), and predominately suction ventilation. Longer anterior slits may aid ventilation by taking in additional water through those openings in anoxic environments or during feeding (Grigg, 1970; Compagno, 1990). In *Heterodontus portjacksoni,* dye directed at the site

of the first gill slit shows that water can be taken in through the first gill slit and expelled through the remaining slits in hypoxic conditions (Grigg, 1970). Yet, in normoxic conditions, water is only taken in through the mouth and expelled from all of the slits (Grigg, 1970). *Heterodontus* feed mainly on crustaceans, which require longer processing times than soft-bodied prey (Gerry et al., 2008), and water may be taken in through the first gill slit to ventilate the other gills during feeding. Unlike other benthic sharks that feed on hard prey, *Heterodontus* does not possess large spiracles that may be used to transport water into the oral cavity for ventilation. Many benthic genera with longer anterior slits feed on crustaceans and/or other complex prey that may require extensive prey processing, such as *Scyliorhinus* (Lyle, 1983; Ebert et al., 1996; Farina and Ojeda, 1993). In these genera, increased spacing between anterior slits may provide room for branchial muscles to function independently of other arches. Most benthic genera have at least one slit over the fin (52% of genera have one over the fin, 41% of genera have two to four over the fin), which would decrease substrate disturbance during suction ventilation by directing more exhaled water over the pectoral fin than the substrate. Less substrate disturbance may also decrease the chances of alerting potential prey or predators, as well as preventing inhalation of debris stirred up from the substrate. Most of the benthic genera with three or four slits over the fin are in the orders Heterodontiformes and Orectolobiformes and are considered to be sluggish swimmers that spend long periods of time resting on the substrate (Compango, 1984). However, *Pristiophorus* has similar length slits and spacing, with no slits over the fin. The only benthic genera that use ram ventilation are the Hexanchiformes, which are slow continual epibenthic swimmers with no slits over fin. The sixgill genera have similar slit lengths and spacing, but the sevengills have decreased slit length and spacing that appears to be due to packing one more pair of branchial arches in the pharynx.

Bathic and micropelagic genera characterize body type 4 and comprise genera solely in the order Squaliformes (Thomson and Simanek, 1977) (Fig. 8D). The pectoral fins are positioned relatively higher on the lateral body wall in "squaloid" sharks compared with other species (Thomson and Simanek, 1977). This may preclude gill slits from being located over the pectoral fin since the slits would have to wrap around below and above the fin, where the fin would be in the way of branchial movements impairing ventilatory function. Most bathic and micropelagic genera have similar slit lengths and spacing, and all ventilate by suction, which may be necessary in these oxygen-low habitats. Furthermore, slits of similar length limit water resistance and thus drag when swimming (Cheer et al., 2001). However, there are some characteristics that differ between the bathic and micropelagic habitats. Bathic body types contain genera in the larger bodied Centrophoridae, Oxynotidae, Somniosidae, and most of the Dalatiidae, whereas micropelagic body types contain smaller bodied genera in Squalidae and Etmopteridae, as well as *Euprotomicrus* and *Isistius* in Dalatiidae. Most bathic genera have similar slit length and spacing; however, several genera have similar slit length and decreased spacing. Increasing gill slit length is only coupled with similar spacing (*Centrophorus*), whereas decreased length is always coupled with decreased spacing (*Scymnodon*). Bathic squaliform genera are not fast swimmers, have little calcification of the skeleton, and have relatively long trunks (Compagno, 1990), where similarly spaced lengths and slits may decrease induced drag. Most bathic genera have large spiracles, which augments suction ventilation by drawing ambient water into the oral cavity (Summers and Ferry-Graham, 2001, 2003). Unfortunately, the biology and behavior of most bathic taxa is unknown, and ecological relationships to body form and gill slit morphology are based on body morphology (Compagno, 1990).

Micropelagic ecomorphotypes of body type 4 examined here comprise genera in

Squalidae and Etmopteridae, as well as *Euprotomicrus* and *Isistius* in Dalatiidae (Fig. 8D). The etmopterid genera and *Euprotomicrus* are epipelagic or mesopelagic small deep-sea sharks, all of which have similar gill slit lengths and spacing, which may function to maximize ventilatory efforts in these oxygen- and food-poor environments. Most squalid genera have a longer fifth slit with decreasing spacing, but some have similar lengths with decreased or similar spacing. Species in the family Squalidae have the largest bathymetric and geographic range of any shark group (Compagno, 1984), and gill slit morphology may mirror that variety. The longer fifth slit of *Squalus* is just anterior to the pectoral girdle and may allow increased exhalation flow when swimming. Again, the presence of large spiracles suggests ventilation by suction as in *Squalus* and *Leucoraja* (Wilga and Motta, 1998; Summers and Ferry-Graham, 2001, 2003). However, little is known about the biology of most of these genera except for *Squalus acanthias*.

Finally, Squatiniformes have a flattened body type with expanded pectoral fins and do not fit nicely within any of the four body types other than being benthic in nature. Similar to other squalean genera, *Squatina* does not have any gill slits over the fin and uses suction to ventilate the gills. However, *Squatina* is the only other genus besides *Centrophorus* that has increasing slit length as well as a shorter last slit space. The shorter last space may be due to the expanded pectoral fins, but the functional reason for increasing slit length is not clear. Squatinid species have large spiracles, which assist water intake during suction ventilation. Expelled water may be directed under the expanded pectoral fins and expelled out the posterior end of the pectoral fins, as observed in some skate and ray species (Wilga et al., 2012).

Evolution of Ventilatory Mode and the Relationship to Gill Slit Morphology

Understanding the evolution of ventilatory mode may further explain trends in the evolution of gill slit morphology. Classification of ventilatory mode for each lineage in this study is based on observations in captive settings, quantification of ventilation mechanics of representative species within each major group, or inferences on ventilatory mode based on habitat and morphology (presence of spiracles and labial folds) (Compagno, 1984). The basal ventilatory mode for modern sharks is likely suction (Fig. 6, dashed white squares). In the galean lineage, suction ventilation is retained in the more basal genera, Heterodontiformes and Orectolobiformes, wherein all but one derived orectolobiform genus (*Rhincodon*) are benthic dwellers (Grigg, 1970; Compagno, 1984). Ram ventilation may assist suction ventilation when swimming, as has been reported for several shark species (Compagno, 1984; Graham et al., 1990; Summers and Ferry-Graham, 2003). A switch to ram ventilation evolved in Lamniformes (Graham et al., 1990), whereas suction ventilation was retained in Carcharhiniformes, except for Carcharhinidae, in which ram and possibly transition ventilation evolved in some genera (Compagno, 1984).

The basal ventilatory mode for Squalea is likely suction ventilation as well, although ram cannot be ruled out. Ram ventilation evolved in Hexanchiformes and Chlamydoselachiformes, the two basal squalean groups (Compagno, 1984; Summers and Ferry-Graham, 2003). Although the ventilation behavior of most squaliform genera is unknown, most have large spiracles, which suggests that suction ventilation is the predominate mode (Compagno, 1984). Pristiophoriformes and Squatiniformes have benthic genera that use suction ventilation and have large spiracles. Thus, two scenarios are possible: 1) suction ventilation is the basal state for modern sharks and was retained in galean and squalean clades, whereas ram ventilation independently evolved in two galean (Lamniformes and Carcharhinidae) and one squalean group (Hexanchiformes), or 2) suction ventilation evolved at the base of the galean clade

(Heterodontiformes and Orectolobiformes), and ram ventilation evolved at the base of the squalean clade (Hexanchiformes), with suction evolving independently in Squaliformes and Carcharhiniformes (Summers and Ferry-Graham, 2003). In either case, the basal morphology for suction-ventilating genera is to have gill slits of similar length and spacing with no slits over the fin, as is basal for modern and extinct shark genera. However, heterodontiform and orectolobiform genera have decreasing slit lengths, some also with decreased spacing with at least three slits over the fin, or both, which could have evolved from the basal suction morphology. Subsequently, a great diversity of gill slit character states then evolved independently in various suction-ventilating genera. The basal state for ram-ventilating genera is also similar lengths and spacing with no slits over the fin, after which various combinations of decreasing length and space and number over the fins evolved (see basal Lamniformes, Carcharhinidae, and Hexanchiformes in Fig. 6) (Compagno, 1984; Graham et al., 1990). Unfortunately, examination of fossil specimens is not helpful in resolving the issue of whether basal neoselachians were suction or ram ventilators, but parsimony indicates that suction ventilation is likely basal.

Hydrodynamic Effects of Gill Slit Morphology

Differences in gill slit morphology may be further understood by exploring the relationships between gill slit morphology and fluid flow patterns inside the pharynx, in parabranchial cavities, and external to the gill slits. Computational fluid dynamic models suggest that volume flow rates are highest at the posterior internal branchial slits in ram suspension–feeding fishes with cylindrical pharyngeal cavities and are highest at the anterior internal slits in conical pharyngeal cavities (Cheer et al., 2001). In that ram suspension feeding and ram ventilation are similar mechanisms, fluid flow patterns inside the pharynx during

ram suspension may provide insight into fluid flow during ventilation (Cech and Cheer, 1994). During ram ventilation, fluid flows continuously into the mouth and through the pharynx and exits through the external gill slits (Cech and Cheer, 1994). If higher flow volumes occur at the anterior internal slits in conical models and at the posterior internal slits in cylindrical models, the external slits at these locations may be longer to accommodate the larger volumes of water. The more derived lamniform genera, *Carcharodon*, *Cetorhinus*, and *Isurus*, appear to have conical pharyngeal cavities (personal observation) and have longer anterior than posterior slits. It is not clear how higher volumes pass through posterior gills that may be decreasing in other sharks with more cylindrical heads. However, it is possible that the last slit may be kept open to serve as an exit valve for excess water flow.

During suction ventilation, water exiting the gill slits creates turbulent flow around the body (Brown and Muir, 1970). As discussed above, most suction-ventilating fishes are benthic dwelling, and turbulent flow exiting the gills could disturb sediment around the head of the fish (Brown and Muir, 1970; Compagno, 1984). In general, two or more posterior slits are positioned over the pectoral fin in most suction-ventilating genera of sharks. Water flowing out of the slits may be turbulent, but slit placement over the pectoral fin likely limits sediment disturbance. Those suction-ventilating genera with none or one pair of slits positioned over the pectoral fin, such as *Squalus*, are typically coastal dwelling and spend more time swimming through the water column than other benthic-dwelling, suction-ventilating genera (Compagno, 1984). No studies have investigated the hydrodynamic effects of water exiting the gill slits during ventilation in resting or swimming sharks. Investigation of pharyngeal dimensions across a broad range of species and fluid flow patterns in the pharynx, in parabranchial chambers, and exiting the gill slits in vivo are necessary to further understand the relationship among

gill slit morphologies, ventilation modes, and fluid dynamics.

Conclusions

Gill slit morphology among the extant shark genera examined here is more diverse than previously expected, with many character states of length, spacing, and position over the fin evolving across the phylogeny. Ecomorphological relationships provide some insight into how gill slit morphology varies by body type and ventilation mode. Morphological variation among the gill slits may be associated with functional differences in slit movements during ventilation. Gill slit placement relative to the pectoral fin may depend more on pectoral fin placement on the body, which appears to be related to habitat. The study of ventilation mechanics in shark species with diverse gill slit character states will provide further insight into the function of gill slit morphology in extant sharks.

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APPENDIX 1.   STANDARDIZED MEANS OF GILL SLIT LENGTH BY GENUS.

Order and Family	Genus	n^a	1	2	3	4	5	6	7
Heterodontiformes									
Heterodontidae	*Heterodontus*	5A	1.000	0.823	0.761	0.618	0.498		
Heterodontidae	*Heterodontus*	4J	1.000	0.874	0.660	0.575	0.407		
Orectolobiformes									
Brachaeluridae	*Brachaelurus*	1J	1.000	1.000	1.000	1.000	1.000		
Ginglymostomatidae	*Ginglymostoma*	1J	1.000	0.920	1.000	0.920	0.830		
Hemiscyllidae	*Chiloscyllium*	6A 4J	0.834	0.881	0.888	0.912	0.753		
Hemiscyllidae	*Hemiscyllium*	1J	0.580	0.580	0.580	0.670	1.000		
Orectolobidae	*Orectolobus*	2A	0.558	0.619	0.577	0.516	1.000		
Rhinidae	*Rhincodon*	1J	1.000	0.890	0.890	0.780	0.670		
Stegostomatidae	*Stegostoma*	4J	0.858	0.938	0.958	0.848	0.825		
Carcharhiniformes									
Carcharhinidae	*Carcharhinus*	5A 20J	0.910	0.939	0.935	0.905	0.706		
Carcharhinidae	*Galeocerdo*	2A	0.976	0.875	0.912	0.912	0.548		
Carcharhinidae	*Glyphis*	1J	0.830	0.850	1.000	0.780	0.600		
Carcharhinidae	*Isogomphodon*	1J	0.940	1.000	0.880	0.810	0.630		
Carcharhinidae	*Loxodon*	3J	0.863	0.933	0.981	0.844	0.796		
Carcharhinidae	*Prionace*	1J	0.910	0.910	0.850	1.000	0.610		
Carcharhinidae	*Rhizoprionodon*	4A	0.916	0.936	0.983	0.912	0.822		
Carcharhinidae	*Rhizoprionodon*	4J	0.809	0.940	0.982	0.924	0.726		
Carcharhinidae	*Scoliodon*	2A	0.875	0.958	0.833	0.917	1.000		
Carcharhinidae	*Triaenodon*	1J	0.800	0.800	0.800	1.000	1.000		
Hemigaleidae	*Hemipristis*	2A	0.976	0.976	0.929	0.833	0.595		
Hemigaleidae	*Paragaleus*	1J	0.880	0.820	1.000	1.000	1.000		
Proscyllidae	*Eridacnis*	3A	1.000	0.933	0.800	0.667	0.422		
Proscyllidae	*Proscyllium*	3A	1.000	0.789	0.681	0.715	0.470		
Scyliorhinidae	*Apristurus*	8A	0.848	0.930	0.921	0.773	0.546		
Scyliorhinidae	*Atelomycterus*	3A	1.000	0.944	0.889	0.883	0.589		
Scyliorhinidae	*Cephaloscyllium*	3A	0.967	1.000	0.907	0.851	0.656		
Scyliorhinidae	*Cephaloscyllium*	1J	0.900	1.000	0.900	1.000	0.500		
Scyliorhinidae	*Cephalurus*	1J	1.00	0.94	1.00	0.67	0.56		
Scyliorhinidae	*Galeus*	15A	0.907	0.919	0.913	0.792	0.513		
Scyliorhinidae	*Haelaelurus*	3A	1.000	0.806	0.722	0.639	0.389		
Scyliorhinidae	*Haploblepharus*	3A	0.970	0.915	0.880	0.714	0.676		
Scyliorhinidae	*Parmaturus*	2A	0.714	1.000	0.914	0.768	0.635		
Scyliorhinidae	*Poroderma*	2A	1.000	0.679	0.659	0.458	0.335		
Scyliorhinidae	*Schroedericthyes*	4A	1.000	0.867	0.735	0.633	0.429		

Order and Family	Genus	n^a	1	2	3	4	5	6	7
Scyliorhinidae	*Scyliorhinus*	9A	1.000	0.794	0.757	0.625	0.464		
Scyliorhinidae	*Scyliorhinus*	2J	1.000	0.944	0.683	0.633	0.369		
Sphyrnidae	*Sphyrna*	9J	0.912	0.894	0.933	0.825	0.638		
Triakidae	*Galeorhinus*	2J	1.000	1.000	0.892	0.925	0.700		
Triakidae	*Hemitriakis*	3J	0.759	0.824	0.870	0.852	0.833		
Triakidae	*Mustelus*	16A	0.941	0.944	0.951	0.869	0.591		
Triakidae	*Mustelus*	8J	0.840	0.827	0.909	0.838	0.605		
Triakidae	*Triakis*	9J	1.000	0.978	0.919	0.845	0.624		
Lamniformes									
Mitsukurinidae	*Mitsukurina*	1J	1.000	0.700	0.700	0.700	0.700		
Odontaspididae	*Odontaspis*	1J	0.961	0.981	0.961	0.961	1.000		
Alopidae	*Alopias*	3J	0.829	0.988	0.944	0.897	0.878		
Cetorhinidae	*Cetorhinus*	1J	1.000	0.930	0.930	0.947	0.947		
Lamnidae	*Isurus*	2J	0.983	0.793	0.769	0.769	0.798		
Lamnidae	*Carcharodon*	1J	1.000	0.941	0.882	0.941	0.941		
Hexanchiformes									
Chlamydoselachidae	*Chlamydoselachus*	2A	0.938	0.952	0.795	0.677	0.718	0.761	
Hexanchidae	*Hexanchidae*	3J	1.000	0.757	0.729	0.700	0.786	0.686	
Hexanchidae	*Heptranchias*	1J	1.000	0.944	0.889	0.833	0.833	0.789	0.722
Hexanchidae	*Notorynchus*	4J	1.000	0.910	0.862	0.757	0.705	0.575	
Squaliformes									
Centrophoridae	*Centrophorus*	3A	0.677	0.735	0.768	0.952	1.000		
Centrophoridae	*Centrophorus*	3J	0.748	0.748	0.767	0.870	1.000		
Centrophoridae	*Deania*	2J	0.912	0.941	0.857	0.929	0.828		
Dalatiidae	*Centroscyllium*	5A 5J	0.930	0.895	0.923	0.884	0.864		
Dalatiidae	*Dalatias*	2J	0.974	0.974	0.947	0.921	1.000		
Dalatiidae	*Etmopterus*	14A	0.98	0.854	0.831	0.858	0.859		
Dalatiidae	*Etmopterus*	4J	1.000	0.850	0.825	0.867	0.783		
Dalatiidae	*Euprotomicrus*	2A	0.750	0.750	0.875	1.000	1.000		
Dalatiidae	*Isistius*	2A	1.000	1.000	1.000	1.000	0.833		
Dalatiidae	*Oxynotus*	3A	0.889	0.886	0.976	0.978	0.627		
Dalatiidae	*Scymnodon*	6A 1J	0.976	0.837	0.794	0.749	0.798		
Dalatiidae	*Squaliolus*	1J	1.000	1.000	1.000	1.000	1.000		
Echinorhinidae	*Echinorhinus*	1J	0.75	0.75	0.83	0.83	1.00		
Etmopteridae	*Aculeola*	1J	1.00	0.75	0.75	0.75	0.75		
Somniosidae	*Centroscymnus*	6J	0.987	0.867	0.867	0.784	0.856		
Somniosidae	*Zameus*	1J	1.00	0.67	0.67	0.50	0.58		
Squalidae	*Cirrhigaleus*	1J	0.63	0.63	0.63	0.63	1.00		
Squalidae	*Squalus*	10A	0.686	0.699	0.712	0.715	1.000		
Squalidae	*Squalus*	2J	0.646	0.646	0.646	0.688	1.000		
Pristiophoriformes									
Pristiophoridae	*Pristiophorus*	2A	0.860	0.900	0.902	0.940	1.000		
Squatiniformes									
Squatinidae	*Squatina*	7J	0.801	0.878	0.877	0.951	0.939		

[a] Juveniles (J) and adults (A) are listed separately when they differ (see Table 1). 1-7 indicate gill slit number.

APPENDIX 2.   STANDARDIZED MEANS OF INTER-GILL SLIT DISTANCE AND NUMBER OF SLITS OVER THE PECTORAL FIN BY GENUS.

Order and Family	Genus	n^a	1–2	2–3	3–5	4–5	5–6	6–7	No. of Slits over Fin
Heterodontiformes									
Heterodontidae	*Heterodontus*	5A 4J	0.958	0.816	0.759	0.676			3
Orectolobiformes									
Brachaeluridae	*Brachaelurus*	1J	1.000	0.830	0.750	0.750			2
Ginglymostomatidae	*Ginglymostoma*	1J	1.000	0.510	0.670	0.330			2
Hemiscyllidae	*Chiloscyllium*	6A	1.000	0.933	0.808	0.147			3
Hemiscyllidae	*Chiloscyllium*	4J	0.929	0.895	0.839	0.177			2–3
Hemiscyllidae	*Hemiscyllium*	1J	0.860	0.860	1.000	0.290			3
Orectolobidae	*Orectolobus*	2A	0.955	0.944	0.942	0.616			4
Rhinidae	*Rhincodon*	1J	0.970	1.000	0.790	0.480			2
Stegostomatidae	*Stegostoma*	4J	1.000	0.842	0.669	0.119			3
Carcharhiniformes									
Carcharhinidae	*Carcharhinus*	5A	0.903	0.933	0.847	0.671			1
Carcharhinidae	*Carcharhinus*	20J	0.915	0.889	0.814	0.700			1–2
Carcharhinidae	*Galeocerdo*	2A	1.000	0.940	0.601	0.464			1–2
Carcharhinidae	*Glyphis*	1J	1.000	0.630	1.000	0.750			1
Carcharhinidae	*Isogomphodon*	1J	1.000	0.890	0.780	0.780			1
Carcharhinidae	*Loxodon*	3J	0.944	0.778	0.861	0.694			1–2.5
Carcharhinidae	*Prionace*	1J	1.000	0.760	0.820	0.590			2
Carcharhinidae	*Rhizoprionodon*	4A 4J	0.979	0.935	0.796	0.764			0–2
Carcharhinidae	*Scoliodon*	2A	1.000	0.667	0.583	0.667			1
Carcharhinidae	*Triaenodon*	1J	0.800	1.000	1.000	0.800			1
Hemigaleidae	*Hemipristis*	2A	1.000	0.912	0.941	0.699			1
Hemigaleidae	*Paragaleus*	1J	0.670	0.830	1.000	0.500			1
Proscyllidae	*Eridacnis*	3A	1.000	0.759	0.635	0.468			1
Proscyllidae	*Proscyllium*	3A	0.917	0.952	0.786	0.729			1
Scyliorhinidae	*Apristurus*	8A	0.975	0.842	0.693	0.608			0–1
Scyliorhinidae	*Asymbolus*	1J	1.000	0.830	0.670	1.000			1
Scyliorhinidae	*Atelomycterus*	3A	1.000	0.821	0.821	0.685			1
Scyliorhinidae	*Cephaloscyllium*	3A 1J	1.000	0.836	0.771	0.718			2
Scyliorhinidae	*Cephalurus*	1J	1.000	0.880	0.630	0.440			2
Scyliorhinidae	*Galeus*	15A	0.930	0.826	0.803	0.643			1, 2, or 4
Scyliorhinidae	*Haelaelurus*	3A	0.939	0.926	0.828	0.972			1 or 3
Scyliorhinidae	*Haploblepharus*	3A	0.850	0.830	0.905	0.871			2
Scyliorhinidae	*Parmaturus*	2A	0.917	0.875	0.833	0.708			2
Scyliorhinidae	*Poroderma*	2A	0.847	0.915	1.000	0.738			2
Scyliorhinidae	*Schroedericthyes*	4A	1.000	0.813	0.795	0.670			1–2
Scyliorhinidae	*Scyliorhinus*	9A	0.972	0.915	0.872	0.701			1
Scyliorhinidae	*Scyliorhinus*	2J	1.000	1.000	0.739	0.606			1
Sphyrnidae	*Sphyrna*	9J	0.984	0.858	0.754	0.594			0–1
Triakidae	*Galeorhinus*	2J	1.000	1.000	1.000	0.900			1
Triakidae	*Hemitriakis*	3J	1.000	0.690	0.841	0.794			1
Triakidae	*Mustelus*	16A 8J	0.970	0.843	0.836	0.730			1
Triakidae	*Triakis*	9J	0.990	0.888	0.810	0.579			1–2
Lamniformes									
Mitsukurinidae	*Mitsukurina*	1J	0.920	1.000	1.000	1.000			0
Odontaspididae	*Odontaspis*	1J	1.000	0.830	0.830	0.500			0
Alopidae	*Alopias*	3J	0.958	0.978	0.826	0.460			2
Cetorhinidae	*Cetorhinus*	1J	1.000	1.000	0.870	0.330			0
Lamnidae	*Isurus*	2J	1.000	0.646	0.536	0.268			0
Lamnidae	*Carcharodon*	1J	0.880	1.000	1.000	0.590			0
Hexanchiformes									
Chlamydoselachidae	*Chlamydoselachus*	2A	1.000	0.800	0.900	0.683	0.667		0

Order and Family	Genus	n^a	1–2	2–3	3–5	4–5	5–6	6–7	No. of Slits over Fin
Hexanchidae	*Hexanchidae*	3J	0.750	0.583	0.583	1.000	0.583		0
Hexanchidae	*Heptranchias*	1J	1.000	0.570	0.570	1.000	0.710	0.430	0
Hexanchidae	*Notorynchus*	4J	1.000	0.832	0.761	0.705	0.614	0.503	0
Squaliformes									
Centrophoridae	*Centrophorus*	3A	0.929	0.805	0.632	0.588			0
Centrophoridae	*Centrophorus*	3J	1.000	0.900	0.720	0.475			0
Centrophoridae	*Deania*	2J	1.000	0.875	0.875	0.550			0
Dalatiidae	*Centroscyllium*	5A	0.933	0.803	0.794	0.553			0
Dalatiidae	*Centroscyllium*	5J	0.943	0.865	0.967	0.741			0
Dalatiidae	*Dalatias*	2J	1.000	0.957	0.714	0.393			0
Dalatiidae	*Etmopterus*	14A	0.988	0.878	0.827	0.746			0
Dalatiidae	*Etmopterus*	4J	1.000	0.887	0.754	0.787			0
Dalatiidae	*Euprotomicrus*	2A	1.000	1.000	1.000	0.750			0
Dalatiidae	*Isistius*	2A	1.000	1.000	1.000	0.775			0
Dalatiidae	*Oxynotus*	3A	1.000	0.783	0.694	0.703			0
Dalatiidae	*Scymnodon*	6A 1J	0.984	0.727	0.746	0.606			0
Dalatiidae	*Squaliolus*	1H	1.000	1.000	1.000	1.000			0
Echinorhinidae	*Echinorhinus*	1J	1.000	1.000	0.670	0.670			0
Etmopteridae	*Aculeola*	1J	1.000	0.770	0.620	0.540			0
Somniosidae	*Centroscymnus*	6J	0.948	0.972	0.873	0.887			0
Somniosidae	*Zameus*	1J	1.000	1.000	1.000	0.710			0
Squalidae	*Cirrhigaleus*	1J	1.000	1.000	1.000	0.600			0
Squalidae	*Squalus*	10A 2J	0.988	0.827	0.781	0.611			0
Pristiophoriformes									
Pristiophoridae	*Pristiophorus*	2A	1.000	0.875	0.750	0.375			0
Squatiniformes									
Squatinidae	*Squatina*	7J	0.929	0.705	0.667	−0.142			0

[a] Juveniles (J) and adults (A) are listed separately when they differ (see Table 1). Numbers 1-7 indicate slit number.

APPENDIX 3.   RESULTS OF ANALYSIS OF VARIANCE AMONG GILL SLIT LENGTHS BY GENUS.

Order and Family	Genus	*F/H** Statistic	*P* value	Stage[a]	State[b]	MCT[c]
Heterodontiformes						
Heterodontidae	*Heterodontus*	54.736	<0.001	A	D	1>23>45
Heterodontidae	*Heterodontus*	26.328	<0.001	J	D	12>3>45
Orectolobiformes						
Hemiscyllidae	*Chiloscyllium*	1.648	0.179	AJ	S	
Orectolobidae	*Orectolobus*	5.283*	0.26	A	S	
Stegostomatidae	*Stegostoma*	1.170	0.363	J	S	
Carcharhiniformes						
Carcharhinidae	*Carcharhinus*	39.1026*	<0.001	AJ	S5	1234>5
Carcharhinidae	*Galeocerdo*	4.938*	0.294	A	S	
Carcharhinidae	*Loxodon*	0.629	0.653	J	S	
Carcharhinidae	*Rhizoprionodon*	1.388	0.301	A	S	
Carcharhinidae	*Rhizoprionodon*	3.437	0.035	J	D2	3>5
Carcharhinidae	*Scoliodon*	3.702*	0.448	A	S	
Hemigaleidae	*Hemipristis*	7.959*	0.093	A	S	
Proscyllidae	*Eridacnis*	3.506	0.049	A	D2	1>5
Proscyllidae	*Proscyllium*	15.581	<0.001	A	L1	1>2345
Scyliorhinidae	*Apristurus*	6.255	<0.001	A	D2	123>5
Scyliorhinidae	*Atelomycterus*	13.855	<0.001	A	S5	1234>5
Scyliorhinidae	*Cephaloscyllium*	3.424	0.052	AJ	S	
Scyliorhinidae	*Galeus*	35.315*	<0.001	A	S5	1234>5
Scyliorhinidae	*Haelaelurus*	31.101	<0.001	A	D	1>23>5
Scyliorhinidae	*Haploblepharus*	5.451	0.014	A	D2	1>45
Scyliorhinidae	*Parmaturus*	7.127*	0.129	A	S	
Scyliorhinidae	*Poroderma*	8.089*	0.088	A	S	
Scyliorhinidae	*Schroedericthyes*	14.14	<0.001	A	D2	12>5
Scyliorhinidae	*Scyliorhinus*	27.750	<0.001	A	D	1>2>4>5
Scyliorhinidae	*Scyliorhinus*	67.049	<0.001	J	D	12>34>5
Sphyrnidae	*Sphyrna*	8.976	<0.001	J	D2	123>5
Triakidae	*Galeorhinus*	7.531*	0.110	J	S	
Triakidae	*Hemitriakis*	0.132	0.967	J	S	
Triakidae	*Mustelus*	52.326*	<0.001	A	S5	1234>5
Triakidae	*Mustelus*	12.531*	0.014	J	D2	3>5
Triakidae	*Triakis*	27.441*	<0.001	J	D2	123>5
Lamniformes						
Alopidae, Odontaspididae	*Alopias, Odontaspis*	7.552*	0.109	J	S	
Mitsukurinidae, Cetorhinidae, Lamnidae	*Mitsukurina, Cetorhinus, Carcharodon, Isurus*	12.181*	0.0016	J	L1	
Hexanchiformes						
Chlamydoselachidae	*Chlamydoselachus*	7.582*	0.181	A	S	
Hexanchidae	*Hexanchidae*	6.580*	0.254	J	S	
Hexanchidae	*Hepranchias, Notorynchus*	33.750	<0.001	J	D	1>23>34 >45>56>67
Squaliformes						
Centrophoridae	*Centrophorus*	22.693	<0.001	A	I2	123<45
Centrophoridae	*Centrophorus*	8.216*	0.084	J	S	
Centrophoridae	*Deania*	1.286	0.864	J	S	
Dalatiidae	*Centroscyllium*	2.570*	0.632	AJ	S	
Dalatiidae	*Dalatias*	1.409*	0.789	J	S	
Dalatiidae	*Etmopterus*	12.551*	0.014	A	D2	1>2
Dalatiidae	*Etmopterus*	1.639	0.216	J	S	

Order and Family	Genus	F/H* Statistic	P value	Stage[a]	State[b]	MCT[c]
Dalatiidae	*Euprotomicrus*	2.625*	0.622	A	S	
Dalatiidae	*Isistius*	4.0*	0.406	A	S	
Dalatiidae	*Oxynotus*	3.177	0.063	A	S	
Dalatiidae	*Scymnodon*	2.959	.036	AJ	D2	1>5
Somniosidae	*Centroscymnus*	1.850	0.151	J	S	
Squalidae	*Squalus*	23.929*	<0.001	A	L5	1234 <5
Squalidae	*Squalus*	4.980*	0.289	J	S	
Pristiophoriformes						
Pristiophoridae	*Pristiophorus*	2.281*	0.684	A	S	
Squatiniformes						
Squatinidae	*Squatina*	3.567	0.017	J	I2	1<45

[a] Juveniles (J) and adults (A) are listed separately when the results of multiple comparisons test (MCT) differ and are combined when similar (AJ).

[b] S, similar lengths among all slits; D, decreasing anterior to posterior with at least three different groups; D2, decreasing anterior to posterior in only two different groups; L, longer followed by slit number that is longer than all others; I2, increasing anterior to posterior in only two groups. S, shorter followed by slit number that is shorter than all others.

[c] For clarity, ties are not shown in the MCT: for example 1<45 = 123<2345.

APPENDIX 4.   RESULTS OF ANALYSIS OF VARIANCE AMONG INTER-GILL SLIT DISTANCES BY GENUS.

Order and Family	Genus	F/H* Statistic	P value	Stage[a]	State[b]	MCT[c]
Heterodontiformes						
Heterodontidae	*Heterodontus*	2.745	0.077	AJ	S	
Orectolobiformes						
Hemiscyllidae	*Chiloscyllium*	18.725*	<0.001	A	D2	12>4
Hemiscyllidae	*Chiloscyllium*			J	S4	123>4
Orectolobidae	*Orectolobus*	4.623*	0.267	A	S	
Stegostomatidae	*Stegostoma*	43.012	<0.001	J	D	1>3>4
Carcharhiniformes						
Carcharhinidae	*Carcharhinus*	2.818	0.072	A	S	
Carcharhinidae	*Carcharhinus*	55.676	<0.001	J	D2	12>4
Carcharhinidae	*Galeocerdo*	8.369*	0.079	A	S	
Carcharhinidae	*Loxodon*	2.628	0.122	J	S	
Carcharhinidae	*Rhizoprionodon*	1.809	0.199	AJ	S	
Carcharhinidae	*Scoliodon*	3.068*	0.381	A	S	
Hemigaleidae	*Hemipristis*	5.155*	0.210	A	S	
Proscyllidae	*Eridacnis*	10.966	0.003	A	L1	1>234
Proscyllidae	*Proscyllium*	1.285	0.344	A	S	
Scyliorhinidae	*Apristurus*	13.276*	0.004	A	D2	1>34
Scyliorhinidae	*Atelomycterus*	9.900*	0.019	A	D2	1>4
Scyliorhinidae	*Cephaloscyllium*	5.758*	0.124	AJ	S	
Scyliorhinidae	*Galeus*	22.861*	<0.001	A	D2	12>4
Scyliorhinidae	*Haelaelurus*	0.749	0.553	A	S	
Scyliorhinidae	*Haploblepharus*	3.620	0.065	A	S	
Scyliorhinidae	*Parmaturus*	3.994*	0.362	A	S	
Scyliorhinidae	*Poroderma*	4.562*	0.210	A	S	
Scyliorhinidae	*Schroedericthyes*	12.652	0.005	A	D2	1>4
Scyliorhinidae	*Scyliorhinus*	4.641	0.008	A	D2	1>4
Scyliorhinidae	*Scyliorhinus*	6.811*	0.067	J	S	
Sphyrnidae	*Sphyrna*	10.132	<0.001	J	D2	12>4
Triakidae	*Galeorhinus*	3.00	0.857	J	S	
Triakidae	*Hemitriakis*	3.022	0.094	J	S	
Triakidae	*Mustelus*	31.971*	<0.001	AJ	L1	1>234
Triakidae	*Triakis*	26.333*	<0.001	J	D2	12>4
Lamniformes						
Alopidae	*Alopias*	6.423	0.016	J	D2	12>4
Lamnidae, Mitsukurinidae	*Isurus, Mitsukurina*	2.950 *	0.399	J	S	
Odontaspididae, Cetorhinidae, Lamnidae	*Odontaspis, Cetorhinus, Carcharodon*	6.834	0.013	J	S4	123>4
Hexanchiformes						
Chlamydoselachidae	*Chlamydoselachus*	4.763*	0.312	A	S	
Hexanchidae	*Hexanchidae*	4.163	0.384	J	S	
Hexanchidae	*Heptranchias, Notorynchus*	13.679*	0.018	J	D2	1>56
Squaliformes						
Centrophoridae	*Centrophorus*	3.253	0.081	A	S	
Centrophoridae	*Centrophorus*	18.518	<0.001	J	D2	1>34
Centrophoridae	*Deania*	5.178	0.267	J	S	
Dalatiidae	*Centroscyllium*	5.858	0.007	A	S4	123>4
Dalatiidae	*Centroscyllium*	2.663	0.083	J	S	
Dalatiidae	*Dalatias*	6.189*	0.038	A	S4	123>4
Dalatiidae	*Etmopterus*	22.858*	<0.001	A	D2	1>34
Dalatiidae	*Etmopterus*	2.601	0.100	J	S	
Dalatiidae	*Euprotomicrus*	3.000*	0.857	A	S	
Dalatiidae	*Isistius*	6.857*	0.343	A	S	

APPENDIX 4.　CONTINUED.

Order and Family	Genus	*F/H** Statistic	*P* value	Stage[a]	State[b]	MCT[c]
Dalatiidae	*Oxynotus*	3.752	0.060	A	S	
Dalatiidae	*Scymnodon*	14.019*	0.003	AJ	D2	1>4
Somniosidae	*Centroscymnus*	2.321*	0.508	J	S	
Squalidae	*Squalus*	28.399	<0.001	AJ	D	12>23>34
Pristiophoriformes						
Pristiophoridae	*Pristiophorus*	6.557*	0.038	A	S	
Squatiniformes						
Squatinidae	*Squatina*	18.795*	<0.001	J	S4	123>4

[a] Juveniles (J) and adults (A) are listed separately when the results of multiple comparisons test (MCT) differ and are combined when similar (AJ).
[b] S, similar distance among all slit interspaces; D, decreasing anterior to posterior with at least three different groups; D2, decreasing anterior to posterior in only two different groups; L, longer followed by slit number that is longer than all others; S, shorter followed by slit number that is shorter than all others.
[c] For clarity, ties are not shown in the MCT (e.g., 1>4 = 123<234).